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USING THE BIOPROTECTION CULTURE FOR DRY FERMENTED SALAMI - THE CONTROL MEASURE OF *Listeria monocytogenes* GROWTH

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Abstract

The control of Listeria monocytogenes (Lm) growth in dry fermented salami represents an important issue in food safety for the meat processing industry. The dry fermented salami represent ready to eat meat products (RTE) with a long shelf life. According to the Regulation (EC) no.2073/2005, Lm represents a food safety criteria for RTE products . Lm is the pathogen agent of human listeriosis, an emerging global zoonoses. The human listeriosis is one of the most severe food born disease that affects certain risk categories in the human population, mainly transmitted during consumption of contaminated food. The purpose of our study is to evaluate the effect of bioprotection cultures for dry fermented salami to control the Lm growth. The bioprotection cultures contain strains of Pediococcus acidilactici that produces pediocin, a bacteriocin with antimicrobial action against Gram positive bacteria, including Lm. The Lm counts during fermentation-smoking and ripening-drying stages highlights that the Lm number decreased by 3.2 log cfu/g during the 30 days in the batch LII , whereas the reduction in the batch LI (without bioprotection culture - the control batch) was 1,03 log cfu/g.Based on results, the use of bioprotection cultures is a useful measure to control the Lm growth for the dry fermented salami. It represents a preventive measure of human listeriosis during the food consumption.

Key words*: bioprotection culture, dry fermented salami, Lm, Pediococus acidilactici, RTE.*

INTRODUCTION

Listeriosis, zoonotic disease produce by *Listeria monocytogenes* (Lm), recognized a notable growing trend during the last two decades in the socio-economic context development of the human population. The consumption of contaminated food, especially ready-to-eat food, is often identified as the cause. Human listeriosis may affect some of the risk category that includes pregnant women, neonates, immunocompromised patients, and the elderly and it causes pathologies such as meningitis, septicemia, encephalitis and abortions. Lm is responsable of a severe pathologies at these risk categories of human population and it can clinically evolve as meningitis, septicemia, encephalitis and abortions (Vázquez-Boland et al., 2001; Di Pinto et al., 2010; Lopez-Valladares et al., 2018). Several factors, such as the infectious dose, the pathogenic features of Lm ingested with food and susceptibility, health and immunological status of the patients play important roles in relation with clinical presentation of disease (Vázquez-Boland et al., 2001). An important characteristhic of Lm is the capacity to survive inside of infected organism cells , for a long period of time, what makes antimicrobial therapy ineffective and assure the protection against the immune response of host (Caplan et al., 2010). The levels of food contamination as low as 10^2 to 10^4 LMO/g of have been associated with human listeriosis (Farber et al., 1991; McLauchlin, 1991).

According to EFSA and ECDC reports «Listeriosis affected around 2,200 people in 2015, causing 270 deaths – the highest number ever reported in the EU. The proportion of cases in the over 64 age group has steadily increased from 56% in 2008 to 64% in 2015. In addition, during this period, the number of reported cases and their proportion in the over 84s has almost doubled." The European Union One Health 2020 Zoonoses Report shows that listeriosis was the fifth, most frequently reported zoonosis, evolving in humans, at the level of the European Union.

Listeria monocytogenes (Lm) is currently known as one of the transmissible zoonotic agents, mainly through food consumption,

being considered a high-risk microbiological hazard in relation to food safety.

Lm is a ubiquitous bacteria, wide spreads in the environment, being present in water, soil and on various objects. The high resistance of Lm is worth mentioning, being a psychrotrophic microorganism that grows in temperature conditions between 0-45 degrees C. The storage temperature range is between 2-10°C in most ready-to-eat products (RTE), which allows the bacteria to multiply. The growth capacity of Lm during shelf life of RTE, makes this pathogen one of food safety criterion, according to the Regulation (EC) no. 2073/2005 regarding the microbiological criteria for food products. The contamination of food products, frequently incriminated as a source of human infection, is caused by either the use of contaminated animal product and/or contaminated and/or recontaminated through the production environment, on the processing flow, due to deficiencies in good practice and good hygienic practice. There is a wide variety of foodstuffs have been reported as source of infection for humans by consumption (e.g., meat products, soft cheeses, dairy products, smoked fish, and salads) but they were usually refrigerated ready-to-eat products (McLauchlin et al., 1990; Farber et al., 1991; Rocourt, 1996). *Listeria monocytogenes* is widespread in the environment and is a potential risk because there is a wide variety of foodstuffs, including the dry fermented sausages, that may be contaminated with LMO (Meloni, 2015). The contamination of foodstuffs recognises various sources, as result of the use of contaminated raw material (i.e., contaminated raw meat for dry feremented products) and/or contamination through the processing environment. The raw meat can be contaminated often, the primary source being the infected animal. Lm could survive and multiply during the fermentation, ripening and drying stages of the dry fermented salami manufacturing. The important resistance of Lm represents the main issue, being a psychrotrophic bacteria and resistant to action of physical and chemical factors. The bacteria can survive and can be grown at a wide range of ph value and a wide range in temperature. The Lm number can be reduced during the manufacturing due the presence of lactic acid, the low value of pH, but an important number of Lm could survive (Farber et al., 1988; Junttila et al., 1989).

The efficiency of bioprotection culture against Lm has been showed by several scientific papers (Korshidian et al., 2021; Aymerich et al., 2019; Saraoui et al., 2018; Bošković et al., 2017 ; Garriga et al., 2015; Winkelströter et al., 2015).

The using of bioprotection culture that contain strains of Pediococcus acidilactici is efficient to inhibit or limit the growth of LMO in dry or semi-dry fermented salami below 26ºC, during the manufacturing stages.

Pediococcus acidilactici produces the pediocin, a bacteriocin with antimicrobial action against Lm. The pediocins are natural bacteriocines produced by several species of *Pediococcus* (i.e., *P. acidilactici*, *P. cellicola*, *P. damnosus*, *P. inopinatus*, *P. pentosaceus*, etc.) (Tomé, et al., 2008; Haakensen et al., 2009). These bacteriocines present an important antimicrobial action against Gram- positive microorganisms, especially pathogenic bacteria.

The purpose of our study is to evaluate the effect of bioprotection cultures for dry fermented salami to control the Lm growth during the manufacturing process.

MATERIALS AND METHODS

In order to asses the behaviour of Lm during the manufacturing process of dry fermented salami under action of bioprotection culture6.1×10^7cfu/g(B-LC-20 SafePro^{TM}, Chr. Hansen) a trial was contucted to count the Lm during the ripening and drying stages. For this purpose, two experimental lots (LI and LII) of dry fermented salami (below 26ºC) were manufactured, based on the Banatean salami recipe and technology, such as: choper of pork and beef meat, mixing of all ingredients, stuffing the meat mixture, fermentation-smoking (alternatives stages of fermentation and smoking), ripening-drying stages. The duration of Banatean salami production process is 30 days. The manufacturing of dry fermented salami requires microbial fermentation produced by starter culture 5×10^6 cfu/g (BFL-F02®, Chr. Hansen). The starter culture is intended for the dry fermented salami to ensure the characteristics of the meat mixture during the fermentation stage (the acidification

process). Both lots contain the starter culture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* (BFL-F02, Chr. Hansen) that is added during the mixing stages, according the technical specification BFL-F02 ver. 4PI-EU-RO 21.08.2012. Each lot has been inoculated with Lm strains directly to the meat recipe, into the bowl chopper. For the artificial inoculation there were used two strains of *Listeria monocytogenes*. The assumed level of contamination was about 1000 cfu/g of sample. The fermentation-smoking stage has been done during 7 days, at 22-24°C in a relative humid air of 85-95%. The ripening and drying stage took place under controlled conditions in temperature (14-15°C) and a humidity of the air raging between 73-75%, until day 30 of the manufacturing process.

LI of Banatean salami with starter culture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* (BFL-F02,Chr. Hansen).

LII of Banatean salami with starter culture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* (5×10^6 ufc/g)) (BFL-F02,Chr. Hansen) and bioprotection culture of *Pediococcusacidolactici*(B-L20SafePro®,Chr. Hansen).

The Lm counts was done during the fermentation-smoking and ripening - drying stages, on 25 g sample on 5 time intervals, such as T0 (day 0), T1 (day 3) , T2 (day 7), T3 (day 14) and T4 (day 30), to counting of Lm on the two lots artificial inoculated. There were tested three inoculated samples (salami stick)/time intervals/lot.

The evaluation of Lm growing has been carried out as a challenge test on the two lots of Banatean salami during the fermentation-smoking and ripening-drying stages. Method of Lm counts is done by the most probable number, using the isolates on Ottaviani & Agosti (incubation the Petri at 37°C, 24 h) and identify 1 to 5 typical colonies using the conventional tests described in the standardized methods ISO 11290-2:2017.

During the fermentation-smoking and ripening-drying stages a complementary test on three samples of Banatean salami (salami stick/each lot/interval of time) was carried out to determine the pH value (measurement by potentiometric method), in five time intervals : T0, T1, T2, T3 and T4.

The results of Lm counts are exprimed as log cfu/g.

RESULTS AND DISCUSSIONS

At the first assessment, the Lm counts registered similar results, with an average of 3.34 log cfu/g for LI and 3.52 log cfu/g for LII. At the end of the fermentation process it is observed an important reduction of Lm number for both batches, 2.64 log cfu/g for LI and 2.04 logcfu/g for LII at T1.The significant decrease carried on for the batch LII, at T2 Lm number being 1.56 log cfu/g. For batch LI, is observed o slightly increase at T2, the Lm number being 3.15 log cfu/g. It can be noted a slight decrease for batch LI, being registered 2.44 log cfu/g at T3. Concerning the batch LII, continued reduction of Lm number, being recorded 1.16 log cfu/g at T3. A significant difference of Lm number is observed between batches at the end of manufacturing production(day 30), i.e for the batch LI being registered 1.89log cfu/g compared to the batch LII where is 0.33 log cfu/g.

Table 1. Results of Lm obtained on LI* of Banatean salami

Sample	day	Log cfu/g	Average of Lm Log cfu/g
1	T0	3.37	3.34±0.03
2		3.35	
3		3.30	
1	T1	2.63	2.64±0.03
2		2.68	
3		2.62	
1	T2	3.18	3.15±0.03
2		3.15	
3		3.12	
1	T3	2.47	2.44±0.03
2		2.40	
3		2.45	
1	T4	1.86	1.89±0.03
2		1.89	
3		1.92	

*LI is the experimental lot of Banatean salami (LI) with the starter culture of *Pediococcus pentosaceus* and *Staphylococcus carnosus* 5×106 ufc/g) (BFL-F02, Chr. Hansen)

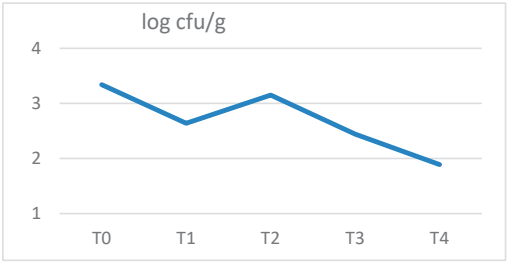

Figure 1. Lm counts in batch LI

Table 2. Results of Lm obtained on LII* of Banatean salami

Sample	day	Log cfu/g	Average of Lm log cfu/g
1	T0	3.57	3.52±0.04
2		3.52	
3		3.48	
1	T1	2.08	2.04±0.03
2		2.04	
3		2.02	
1	T2	1.60	1.56±0.04
2		1.57	
3		1.52	
1	T3	1.20	1.16±0.03
2		1.14	
3		1.16	
1	T4	0.38	0.33±0.04
2		0.30	
3		0.32	

*LII is the experimental lot of Banatean salami with the starter culture *Pediococcus pentosaceus* and *Staphylococcus carnosus* (BFL-F02, Chr. Hansen) and the bioprotection culture *Pediococcus acidolactici* culture (SafePro® B-LC-20, Chr. Hansen Holding)

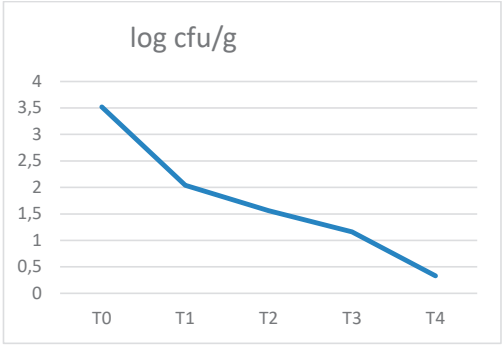

Figure 2. Lm counts in batch LII

Based on the results obtained per each lot of Banatean salami, during fermatation-smoking and ripening-drying stages, the Lm number decreased by 3.2 log cfu/g during the 30 days in the batch LII , whereas the reduction in the batch LI was 1,03 log cfu/g.The assessment of LII on day 30 proves that the bioprotection culture *Pediococcus acidolactici* (SafePro® B-LC-20, Chr. Hansen) and the starter culture *Pediococcus pentosaceus* plus *Staphylococcus carnosus* (BFL-F02, Chr. Hansen) determine an efficient reduction of Lm number. These results are in accordance with observations of Heller-Stahnke (2005): "… *the use of an adjunct culture such as B-LC-20 provides a unique anti-listerial reduction for fermented sausages since it was found that Pediococcus acidilactici is a strong producer of pediocin (which destroys Listeria monocytogenes) at European fermentation temperatures (<26°C) while not being a strong acidifier at 10 this temperature*" (Heller-Stahnke, 2005).

The results of pH evaluation along the fermentation-smoking and ripening-drying stages, expressed as average of triplicate samples, are available in Tabel 3.

Tabel 3. Results of pH along fermentation-smoking and during the ripening and drying stage (expresed as average)

Lot no.	pH	
	LI	LII
T0	5.95±0.02	5.92±0.02
T1	4.78±0.015	4.73±0.03
T2	4.93±0.02	4.74±0.02
T3	4.98±0.01	4.77±0.01
T4	5.0±0.02	4.88±0.02

It is observed an important decrease of pH in both lots, as result of starter culture action, during the fermentation stage. The researches of Foegeding et al. (1992) proved that, as during the dry fermented sausages manufacturing process, the Lm population can be reduce in condition of pH value less than 4.9 at the end of the fermentation process and along the drying stage. The bacteriocin production enhanced the inhibition rate of Lm growth. The pediocin produced by *Pediococcus acidilactici* has an effective activity against Lm growth, during the fermentation stage and during ripening-drying stage. In case of higher pH value, the pediocin will have an antilisterial effect for the remaining Lm. (Foegeding et al., 1992; Nieto-Lazano et al., 2010).

Another study is the one of Korshidian et al. (2021) regarding antibacterial activity of pediocin and pediocin-producing bacteria against *Listeria monocytogenes* in meat products at a wide range of pH. According this study, the pediocin represents an important biopreservatives categorie for meat processing industry. Mehta et al. (2013) showed the importance of pediocines for food industry thanks of their strong activity against food borne pathogenic agents. Furthermore, the pediocin produced by Pediococcus acidilactici, has been considered to be safe (Mehta et al., 2013).

There is a potential risk of Lm contamination of dry fermented salami from various sources (i.e., raw meat, manufacturing processes, etc.) . The use of starter cultures and ensuring the conform condition of ripening and drying can

reduce the the potential of LMO growth in this type of sausages (Meloni, 2015) and represents an very useful food safety measure for meat processing industry, considering the ubiquitous character of Lm and the multitude of contamination sources.

The study of of Bungenstock et al. (2020) who identified among the 169 collected isolates, two new bacteriocin-producing isolates which have the potential to contribute to product and consumer safety: *Pediococcus pentosaceus* LMQS 331.3 and *Pediococcus acidilactici* LMQS 154.1 represents an encouragement for the realization of future researches (Bungenstock et al., 2020).

CONCLUSIONS

Based on results of the study, the bioprotection culture is able to inhibit the growth of Lm for dry fermented sausages till the end of ripening-drying stage, compared to a control starter culture alone.

The using of bioprotection cultures in our study proved that the *Pediococcus acidolactici* culture (B-LC-20 SafeProTM, Chr. Hansen) has a major contribution to limit the Lm growth during the manufacturing process and to ensure the microbial safety of dry fermented salami.

REFERENCES

Aymerich, T., Rodríguez, M., Garriga, M., & Bover-Cid, S. (2019). Assessment of the bioprotective potential of lactic acid bacteria against *Listeria monocytogenes* on vacuum-packed cold-smoked salmon stored at 8°C. *Food microbiology*, 83, 64–70.

Bhunia, A. K., Johnson, M. C., & Ray, B. (1988). Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici. The Journal of applied bacteriology*, 65(4), 261–268.

Bošković, M., Tadic, V., Đorđević, J., Glisic, M., Lakićević, B., Dimitrijević, M., & Baltić, M. (2017). Effect of starter cultures on survival of Listeria monocytogenes in Čajna sausage. *IOP Conference Series: Earth and Environmental Science, 85*.

Bungenstock, L., Abdulmawjood, A., & Reich, F. (2020). Evaluation of antibacterial properties of lactic acid bacteria from traditionally and industrially produced fermented sausages from Germany. *PloS one*, 15(3), Article e0230345. Retrieved December 18, 2019, from https://dx.doi.org/10.1371%2Fjournal.pone.0230345.

Caplan, M.E., & Mateescu, L.A. (2010). Investigation of *L.monocytogenes*- HEP-2 Cells relationships by culture based and microscopy tools. *Scientific Papers. Series D. Animal Science*, LVI.

Di Pinto, A., Novello, L., Montemurro, F., Bonerba, E., & Tantillo, G. (2010). Occurrence of *Listeria monocytogenes* in ready-to-eat foods from supermarkets in Southern Italy. *The new microbiologica*, 33(3), 249–252.

Farber, J.M., & Losos, J.Z. (1988). *Listeria monocytogenes*: a foodborne pathogen. *CMAJ*, 138(5), 413-418.

Farber, J. M., & Peterkin, P. I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiological reviews*, 55(3), 476–511.

Foegeding, P. M., Thomas, A. B., Pilkington, D. H., & Klaenhammer, T. R. (1992). Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. *Applied and environmental microbiology*, 58(3), 884–890.

Garriga, M., Rubio, R., Aymerich, T., & Ruas-Madiedo, P. (2015). Potentially probiotic and bioprotective lactic acid bacteria starter cultures antagonise the *Listeria monocytogenes* adhesion to HT29 colonocyte-like cells. *Beneficial microbes*, 6(3), 337–343.

Haakensen, M., Vickers, D.M., & Ziola, B. (2009). Susceptibility of Pediococcus isolates to antimicrobial compounds in relation to hop-resistance and beer-spoilage. *BMC Microbiol*., 9, 190. doi: 10.1186/1471-2180-9-190.

Hansen, C. (2012). Technical sheet BFL-F02 ver. 4 PI-EU-RO 21.08.2012

Hansen, C. (2013). Technical sheet B-LC-20 SafePro™, ver. 3 PI-EU-RO 06.02.2013

Heller-Stahnke, M.L. (2005). Method for reducing the content of pathogenic organisms present in food materials. Retrieved from https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2005100614&tab=PCTDESCRIPTION

Lopez-Valladares, G., Danielsson-Tham, M. L., & Tham, W. (2018). Implicated Food Products for Listeriosis and Changes in Serovars of *Listeria monocytogenes* Affecting Humans in Recent Decades. *Foodborne pathogens and disease*, 15(7), 387–397.

Mehta, R., Arya, R., Goyal, K., Singh, M., & Sharma, A. K. (2013). Bio-preservative and therapeutic potential of pediocin: recent trends and future perspectives. *Recent patents on biotechnology*, 7(3), 172–178.

Meloni, D. (2015). Presence of *Listeria monocytogenes* in Mediterranean-Style Dry Fermented Sausages. *Foods*, 4(1), 34–50.

Nieto-Lozano, J.C., Reguera-Useros, J.I., Peláez-Martínez, M.C., Sacristán-Pérez-Minayo, G., Gutiérre Fernández, A.J., & Hardisson de la Torre, A. (2010). The effect of the pediocin PA-1 produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages and frankfurters. *Food control*, 21, 679-685.

Rocourt, J. (1996). Risk factors for listeriosis. *Food Control,* 7, 195–202.

Saraoui, T., Leroi, F., Chevalier, F., Cappelier, J. M., Passerini, D., & Pilet, M. F. (2018). Bioprotective Effect of *Lactococcus piscium* CNCM I-4031 Against

Listeria monocytogenes growth and virulence. *Frontiers in microbiology*, 9, Article 1564. Retrieved 2018, March 06, https://doi.org/10.3389/fmicb.2018.01564.

Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., & Kreft, J. (2001). *Listeria pathogenesis* and molecular virulence determinants. *Clinical microbiology reviews*, 14(3), 584–640.

Winkelströter, L. K., & De Martinis, E. C. (2015). In vitro protective effect of lactic acid bacteria on *Listeria monocytogenes* adhesion and invasion of Caco-2 cells. *Beneficial microbes*, 6(4), 535–542.